Fish meals, fish components, and fish protein hydrolysates as potential ingredients in pet foods

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ABSTRACT: An experiment to determine the chemical composition and protein quality of 13 fish substrates (pollock by-products, n = 5; fish protein hydrolysates, n = 5; and fish meals, n = 3) was conducted. Two of these substrates, salmon protein hydrolysate (SPH) and salmon meal with crushed bones (SMB), were used to determine their palatability as components of dog diets. Pollock by-products differed in concentrations of CP, crude fat, and total AA by 71, 79, and 71%, respectively, and GE by 4.1 kcal/g. Fish protein hydrolysates and fish meals were less variable (approximately 18, 14, and 17%, and 1.4 kcal/g, respectively). Biogenic amine concentrations were much higher in fish protein hydrolysates as compared with pollock by-products and fish meals. Pollock liver and viscera had the highest total fatty acid concentrations; however, red salmon hydrolysate and SMB had the highest total PUFA concentrations (49.63 and 48.60 mg/g, respectively). Salmon protein hydrolysate had the highest protein solubility in 0.2% KOH. Based on calculations using immobilized digestive enzyme assay values, lysine digestibility of fish meal substrates was comparable to in vivo cecectomized rooster assay values and averaged approximately 90.3%. Also, pollock milt, pollock viscera, red salmon hydrolysate, and sole hydrolysate had comparable values as assessed by immobilized digestive enzyme assay and rooster assays. A chick protein efficiency ratio (PER) assay compared SMB and SPH to a whole egg meal control and showed that SMB had high protein quality (PER = 3.5), whereas SPH had poor protein quality (PER value less than 1.5). However, using whole egg meal as the reference protein, both fish substrates were found to be good protein sources with an essential AA index of 1.0 and 0.9 for SMB and SPH, respectively. In the dog palatability experiments, a chicken-based control diet and 2 diets containing 10% of either SPH or SMB were tested. Dogs consumed more of the SPH diet compared with the control, and similar amounts of the SMB and control diets. The intake ratios for each were 0.73 and 0.52, respectively. Salmon protein hydrolysate was especially palatable to dogs. These data suggest that chemical composition and nutritional quality of fish substrates differ greatly and are affected by the specific part of the fish used to prepare fish meals and fish protein hydrolysates.

Key words: fish substrate, palatability, pet food, protein quality

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INTRODUCTION

Fish processing generates more than 1 million metric tons of fish waste per year, most of which could be utilized in animal feed (Hardy, 2003). Currently, the major wastes (heads, viscera, skin, and skeleton) are underutilized and often create disposal problems and environmental concerns. Instead of disposing of these fish products as waste, they can supply high protein feed ingredients and palatability-enhancing agents for use in animal foods (Regenstein et al., 2003). Upgrading or recovery of the edible high-grade protein from these wastes will result in renewed interest in use of fish meals and hydrolysates in animal diets.

The pet food industry traditionally has utilized a wide range of protein sources including meat and bone meals, poultry meals, poultry by-products meals, and soybean meal (Fahey, 2004). Alternative protein sources that have been studied in other animal species, but only to a limited extent in companion animals, include fish by-products and fish protein hydrolysates.

Although the advantages of using fish substrates as alternative protein sources are recognized in the pet food literature (Willard, 1990), the lack of characterization of these products has led to their underutilization

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in commercial pet foods. By expanding the database on compositional analyses of fish substrates and by determining bioavailability, palatability-enhancing traits, and immunomodulatory role, nutritionists will be able to increase use of these alternative ingredients in dietary formulations for pets.

The objective of this study was to assess the potential utilization of fish by-products, fish protein hydrolysates, and fish meals by chemical composition characteristics and protein quality indices. Also, an experiment to determine palatability of 1 hydrolysate and 1 meal, namely, salmon protein hydrolysate (**SPH**) and salmon meal with crushed bones (**SMB**), as ingredients in dog diets was conducted.

MATERIALS AND METHODS

Substrates

Thirteen substrates obtained from the processing of marine fin fish (pollock by-products, n = 5; fish protein hydrolysates, n = 5; and fish meals, n = 3) were used in this study. Five fresh Alaska pollock (*Theragra chalcogramma*) by-products including heads, liver, milt, unutilized roe, and viscera with roe removed were obtained from fish processing plants in Kodiak, AK, frozen until freeze dried, and stored in a freezer (–20°C) at the Fishery Industrial Technology Center in Kodiak, AK. They were not stabilized with ethoxyquin. One of the 3 fish meals included white fish meal (**WFM**), a fish protein source produced by cooking, pressing, drying, and milling fresh raw fish; it was obtained from the Kodiak Fish Meal Company (Kodiak, AK) and was stabilized with ethoxyquin. Fish protein and marine oil (Pro Mega) containing unhydrolyzed, intact protein, was obtained from BioOregon Inc. (Warreton, OR), and SMB was obtained from Green Earth Industries (Dulles, VA).

Hydrolysates are proteolytic digests of fish parts remaining after filleting, which are made by subjecting the raw materials to controlled proteolytic enzyme digestion that hydrolyzes the protein into peptides and free AA. Four of the 5 hydrolysates included sole, pink salmon, red salmon, and pollock late season by-products and were obtained from commercial fish processing plants in Kodiak, AK, and frozen until made into hydrolysate. By-products were thawed, cooked, deboned, and the liquid was decanted. The remaining mince was hydrolyzed using a commercial papin to which 0.01% ethoxyquin had been added. After hydrolysis, the proteolytic enzyme was heat inactivated and the hydrolysate dried by evaporation to 60% moisture and then drum-dried to 5% moisture, and the dried products stored under refrigeration (–4°C) until analyzed. The fifth hydrolysate was SPH obtained from Green Earth Industries (Dulles, VA) using a proprietary process.

Chemical Analyses

Each substrate was analyzed for DM, ash (AOAC, 1985), and CP (AOAC, 1995) using a Leco Nitrogen/Protein Determinator (model FP-2000, Leco Corporation, St. Joseph, MI), and for crude fat (AOAC, 1995), GE (Parr Instrument Co., Moline, IL; Parr Instrument Manuals), AA (Beckman 6300 amino acid analyzer, Beckman Coulter Inc., Fullerton, CA, AOAC, 1995), minerals (University of Missouri Experiment Station Chemical Laboratories, AOAC, 1995), biogenic amines (Flickinger et al., 2003), and long chain fatty acids (Lepage and Roy, 1986). All ingredients were analyzed in duplicate, with a 5% error allowed between duplicates; otherwise, the analyses were repeated. Samples of the dog palatability diets were also analyzed for DM, ash (AOAC, 1985), CP (AOAC, 1995), and GE (Parr Instrument Co.; Parr Instrument Manuals). In addition, acid hydrolyzed fat (AACC, 1983; Budde, 1952) was measured instead of crude fat.

Biological Analyses

A protein solubility in potassium hydroxide assay (Araba and Dale, 1990) and the immobilized digestive enzyme assay (**IDEA**) of Schasteen et al. (2002) were conducted on each substrate.

Protein Efficiency Ratio Assay

Sixty 8-d-old female chicks (New Hampshire × Columbian cross) were utilized. The average initial BW/chick was 89.3 ± 0.6 g. Chicks were housed in groups of 5 in starter batteries with raised wire floors in an environmentally regulated room. The protein efficiency ratio (**PER**) assay has been used extensively in animal nutrition studies to evaluate various grain and vegetable proteins (Willis and Baker, 1980; Parsons et al., 1983; Han et al., 1987), and animal meals (Johnston and Coon, 1979; Escalona et al., 1986). The PER assay consists of feeding the test ingredient as the sole source of dietary protein in a diet containing only 9 to 10% CP and is calculated as the amount of weight gain per unit of protein consumed (Johnson and Parsons, 1997). This deficient level of protein provides a sensitive test for distinguishing differences in protein quality among ingredients. The 3 diets were formulated to contain nutrient concentrations that met or exceeded NRC (1994) requirements of chicks, except for protein and AA. The diets were whole egg meal (control) and either SPH or SMB, which were added to provide 10% CP (Table 1).

All animal care procedures were conducted under a research protocol approved by the Institutional Animal Care and Use Committee, University of Illinois, Urbana. The experiment was a completely randomized design including 3 treatments with 4 replications and 5 chicks per replication. The chicks were allowed ad libitum access to food and water over the 9-d assay period. The diets were fed as finely ground mash; thus, no sorting of ingredients was possible. Initial and final BW were recorded. Food consumption was recorded for calculation of G:F and PER. Data were analyzed using ANOVA (version 9, SAS Inst. Inc., Cary, NC). Treat-

Table 1. Chick protein efficiency ratio assay diet used to evaluate fish substrates (as-fed basis)

Item	Dietary treatment		
	Whole egg meal	Salmon meal with crushed bones	Salmon protein hydrolysate
Ingredient composition, %			
Cornstarch	45.7	47.6	52.5
Dextrose	22.8	23.8	26.2
Protein sources[1]	20.5	17.5	10.3
Soybean oil	5.0	5.0	5.0
Limestone	1.22	1.22	1.22
Dicalcium phosphate	2.45	2.45	2.45
NaCl	0.50	0.50	0.50
$MgSO_4 \cdot 7H_2O$	0.35	0.35	0.35
K_2CO_3	0.90	0.90	0.90
Purified vitamin mix[2,3]	0.20	0.20	0.20
Mineral mix[3,4]	0.15	0.15	0.15
Purified choline chloride	0.20	0.20	0.20
Ethoxyquin	0.0125	0.0125	0.0125
DL-Tocopheryl acetate	0.002	0.002	0.002
Chemical composition, (calculated)			
CP, %	10.0	10.0	10.0
Ca, %	1.02	2.16	0.98
P, total %	0.63	1.15	0.53

[1]Protein sources (% incorporated into diet) to provide 10% dietary crude protein.

[2]Provided per kilogram of diet: 20 mg of thiamin·HCl; 50 mg of niacin; 10 mg of riboflavin; 30 mg of D-Ca-pantothenate; 0.04 mg of vitamin B_{12}; 6 mg of pyridoxine·HCl; 0.6 mg of D-biotin; 4 mg of folic acid; 2 mg of menadione dimethylpyrimidinol bisulfite; 15 μg of cholecalciferol; 1789 μg of retinyl acetate; and 250 mg of ascorbic acid.

[3]The carrier was cornstarch for the vitamin mix and limestone for the mineral mix.

[4]Provided per kilogram of diet: 75 mg of manganese (as MnO); 75 mg of iron (as $FeSO_4 \cdot H_2O$); 75 mg of zinc (as ZnO); 8 mg of copper (as $CuSO_4 \cdot 5H_2O$); 0.75 mg of iodine (as CaI_2); and 0.3 mg of selenium (as Na_2SeO_3).

ment differences were determined using the LSD calculated from the pooled SEM from the ANOVA. An alpha level less than 0.05 was designated statistically significant.

The protein quality of SMB and SPH also was evaluated based on their AA composition by using the essential AA index (**EAAI**), which is the nth root of the product of the ratios of each essential AA in the fish substrate to that of a reference protein (Murai et al., 1984). The EAAI was calculated using whole egg meal as the reference protein, and the essential AA ratio for each essential AA was expressed as a percentage of the total essential AA including Cys and Tyr (Murai et al., 1984). The chemical score was determined as amount of AA in the sample as a percentage of the total protein divided by adult dog requirement for the respective AA (NRC, 1985) expressed as a percentage of the protein requirement. The lowest value was used as the score. The AA score makes an adjustment for AA composition in evaluating quality of protein and provides a score denoting the most limiting AA; that is, the essential AA in greatest deficit in that protein.

Cecectomized Rooster Experiment

All surgical and animal care procedures were conducted under a research protocol approved by the Institutional Animal Care and Use Committee, University of Illinois, Urbana. A precision-fed cecectomized rooster assay, as described by Sibbald (1979), was conducted to quantify true AA digestibilities of fish substrates. Thirty-nine 50 wk of age Single Comb White Leghorn roosters were utilized. When the birds were 25 wk of age, cecectomy was performed under anesthesia according to the procedure of Parsons (1985). All roosters were given at least 8 wk to recover from surgery before being used in the experiment. All birds were housed individually in cages with raised, wire floors. They were kept in an environmentally controlled room (24°C) and subjected to a 16-h light and 8-h dark photoperiod. Before the beginning of the experiment, feed and water were supplied ad libitum.

Roosters were deprived of feed for 24 h and then crop-intubated and provided approximately 30 g of each of the 13 fish substrates. Each substrate (pollock by-products, n = 5; fish protein hydrolysates, n = 5; and fish meals, n = 3) was fed to 3 roosters. After crop intubation, excreta (urine and feces) were collected for 48 h on plastic trays placed under each cage. The excreta samples then were freeze-dried (Virtis Genesis 25SL Lyophilizer with a Leybold Model D4B TriVac vacuum pump, Gardiner, NY), weighed, and finely ground with a coffee grinder to pass through a 60-mesh screen. Each sample then was analyzed to determine AA concentrations. In

Table 2. Ingredient and chemical composition of diets containing fish protein substrates for palatability experiments with dogs, as-fed basis

Item, %	Dietary treatment: Control (Experiments 1 and 2)	Salmon protein hydrolysate (Experiment 1)	Salmon meal with crushed bones (Experiment 2)
Ingredient composition			
Brewers rice	43.69	45.23	40.96
Poultry by-product meal	35.06	21.32	27.55
Poultry fat	13.98	16.15	14.22
Fish protein substrate	—	10.00	10.00
Beet pulp	4.00	4.00	4.00
Dried egg	2.00	2.00	2.00
KCl	0.50	0.50	0.50
NaCl	0.40	0.40	0.40
Choline chloride	0.13	0.13	0.13
Vitamin premix[1]	0.12	0.12	0.12
Mineral premix[2]	0.12	0.12	0.12
MgO	—	0.03	—
Chemical composition (analyzed)			
DM, %	93.1	93.9	93.6
	DM basis		
OM, %	92.0	93.6	91.7
CP, %	33.0	34.9	34.2
Acid hydrolyzed fat, %	24.6	23.0	21.3
GE, kcal/g	5.3	5.4	5.4

[1]Provided per kilogram of diet: 14.97 KIU of vitamin A; 0.90 KIU of vitamin D_3; 59.88 IU of vitamin E; 0.60 mg of vitamin K; 11.98 mg of thiamin; 9.58 mg of riboflavin; 17.96 mg of pantothenic acid; 44.91 mg of niacin; 11.98 mg of pyridoxine; 0.11 mg of biotin; 0.72 mg of folic acid; and 0.02 mg of vitamin B_{12}.

[2]Provided per kilogram of diet: 12 mg of manganese (as $MnSO_4$); 90 mg of iron (as $FeSO_4$); 12 mg of copper (as $CuSO_4$); 2.4 mg of cobalt (as $CoSO_4$); 120 mg of zinc (as $ZnSO_4$); 1.5 mg of iodine (as KI); and 0.24 mg of selenium (as Na_2SeO_3).

addition, endogenous excretion of AA was measured from additional roosters held without feed throughout the entire 72-h experimental period. True digestibility of AA was calculated using the method described by Sibbald (1979).

Data were analyzed as a completely randomized design using ANOVA (version 9, SAS Inst. Inc.). Treatment differences were determined using the LSD calculated from the pooled SEM from ANOVA. An alpha level less than 0.05 was designated statistically significant.

Dog Palatability Experiments

Two experiments using the same panel of 20 healthy adult dogs were conducted to determine palatability of SPH and SMB. The dogs (10 beagles and 10 pointers) had BW ranging from 7.9 to 32.8 kg. The dogs were housed individually in indoor-outdoor pens, approximately 1.2 × 1.5 m indoors and 1.2 × 3.0 m outdoors, at Kennelwood Inc., Champaign, IL. The dogs had access to the outside area of the kennel once per day for an average of 2 to 4 h, depending on the weather conditions. Three diets were formulated representing a chicken-based control diet and 2 diets containing SPH or SMB. Composition of the diets is presented in Table 2. Diets were formulated to be similar in GE. Dietary ingredients were identical except for replacement of a portion of the poultry by-product meal with SPH or SMB. Diets were fed as an extruded kibble, with the SPH or SMB being incorporated into the mixture before extrusion. The experiments were designed as 2-bowl, free-choice tests, the most common palatability test used in the pet food industry (Griffin, 2003). This design results in the most reliable data (Hutton, 2002). The dogs were on test for 2 d for each experiment, with no time between experiments. All dogs were allowed free access to water.

In Exp. 1, the dogs were offered 1 kg each of the control diet and SPH in separate bowls for 1 h daily for 2 d. In Exp. 2, the dogs were offered 1 kg each of the control diet and SMB in separate bowls for 1 h daily for another 2 d. The placement of the bowls was alternated each day to eliminate any bowl-placement bias. First choice and first approach data were collected. At the end of the hour, any refused food was weighed to determine food intake of each diet. A sample was taken of each diet and frozen at –4°C for subsequent analyses. In preparation for chemical analyses, the diets were ground with dry ice through a 2-mm screen in a Model 4 Wiley Mill (Thomas-Wiley, Swedesboro, NJ). The amount of each diet consumed was calculated by subtracting the food refusal from the amount of food originally offered. The intake ratio (**IR**) was calculated by dividing the grams consumed of the particular fish substrate diet by the total grams consumed of both diets (Spears et al., 2004). The corrected IR was calculated

as the IR minus 0.5, to indicate a diet preference. An IR of 0.5 indicates no preference; therefore, the corrected IR will detect if there was a diet preference significantly different from zero.

Data were analyzed using the mixed model procedure of SAS (SAS Inst. Inc.). The model contained the fixed effect of diet and the random effect of dog. An alpha level less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Dry matter, OM, CP, crude fat, GE, AA, biogenic amine, mineral, and long-chain fatty acid concentrations of fish substrates are presented in Tables 3, 4, 5, and 6. For the pollock by-products tested (Table 3), DM ranged from 88.7 (viscera) to 99% (roe). Organic matter concentrations were highest for liver (99%) and lowest for heads (75.8%). Notable differences in CP concentrations were apparent among samples, ranging from 9.1 to 80.9%. Crude fat varied from 5.4 (roe) to 84.7% (liver). Liver and viscera had the highest crude fat concentrations (84.7 and 46.0%, respectively). Gross energy content ranged from 4.3 to 8.4 kcal/g.

Pollock roe had the highest total essential AA (**TEAA**), total nonessential AA (**TNEAA**), and total AA (**TAA**) concentrations, and liver the lowest (Table 3). Heads were next highest, followed by milt, then viscera. Individual AA concentrations generally followed this same pattern.

The TAA concentrations were lower than CP concentrations for all by-products. Generally, CP concentrations are higher than TAA concentrations. This was due to substrates containing other N-containing compounds that are not protein such as purines, pyrimidines, and biogenic amines. Data indicate that pollock milt and viscera in particular contained a large quantity of N that was not of AA origin.

Biogenic amines occur from microbial decarboxylation of AA such as Arg, His, Lys, Trp, and Tyr. Putrescine, histamine, cadaverine, and tyramine concentrations are indicators of raw fish freshness and serve as quality indicators for fish meal (Opstvedt et al., 2000). Biogenic amine concentrations for all pollock by-products were relatively low, and for most amines, concentrations were zero. Viscera contained more of the amines compared with the other by-products, indicating greater microbial decarboxylation of AA.

The Ca and P concentrations of pollock heads were highest among pollock substrates, 5.58 and 3.63%, respectively, due to the presence of cartilaginous tissue and bones in the head. Iron concentrations ranged from 11.4 to 62.1 ppm. Milt had the highest K concentration (2.09%), and roe the highest Zn concentration (145.6 ppm). Variation in mineral concentrations among pollock by-products was extreme, with high values sometimes being twice those of the low as noted for K and some trace minerals. Processing variables, including drying and concentration procedures, and nutrient elimination techniques (e.g., for fat) may be partially responsible.

For the protein hydrolysates tested (Table 4), DM concentrations ranged from 89.6 to 96.2%. Organic matter values varied from 91 to 97.2%. Crude fat content of most hydrolysates was similar (average, 15%); however, SPH was below average (2.2%). Although the GE content of most samples was relatively consistent, SPH again was below average (4.8 kcal/g).

Salmon protein hydrolysate had the highest CP, TNEAA, and TAA concentrations of all hydrolysates tested. The TEAA concentrations varied from 27.9 to 35.6%. The concentration of TAA again was lower than the CP concentration, indicating the presence of N-containing compounds that are not true protein.

Biogenic amine concentrations were much higher in fish protein hydrolysates as compared with pollock by-products. Sole hydrolysate had the highest histamine concentration (98.51 μg/g) and pollock late hydrolysate the lowest (15.86 μg/g). Salmon protein hydrolysate had the highest tyramine (411.07 μg/g) concentration and pollock late the lowest (87.85 μg/g). Histamine and tyramine are considered antinutritional compounds (Krizek et al., 2004). Salmon protein hydrolysate also had high agmatine, phenylethylamine, and cadaverine concentrations, demonstrating considerable AA decarboxylation activity in this substrate. Pink salmon had the highest spermidine (145.6 μg/g) and red salmon the highest spermine concentrations (116.2 μg/g).

Notable differences in mineral concentrations were observed among test substrates. Salmon protein hydrolysate had the lowest concentration of Ca (0.06%). Pink salmon had the highest P, Fe, and Zn concentrations (1.02%, 688.08 ppm, and 298.29 ppm, respectively). The K concentrations ranged from 0.39 to 1.86%. Perhaps mineral concentrations varied among hydrolysates because of species of fish studied or method of processing the hydrolysate.

Among the fish meal substrates tested (Table 5), DM values were relatively consistent. Organic matter concentrations ranged from 77 to 96.2%. Crude fat content varied from 9.3 to 21.8%. Although the GE content of WFM and SMB was similar, ProMega was higher (6.3 kcal/g). The CP concentrations were similar for ProMega and WFM, whereas SMB had the lowest CP concentration. ProMega had the highest TEAA and TAA concentrations, and SMB the lowest. More TNEAA were present in WFM, but there was little variation among substrates. Again, the concentrations of TAA were lower than CP concentrations.

Biogenic amine concentrations of these substrates were low, although most amines were present. Salmon meal with crushed bones had the highest cadaverine, tyramine, spermidine, and spermine concentrations, demonstrating a considerable degree of decarboxylation of AA. White fish meal had the highest putrescine and histamine concentrations (64.25 and 14.49 μg/g, respectively), and ProMega the lowest (34.71 and 0 μg/g, respectively).

Table 3. Chemical composition of pollock by-products (DM basis)

Item, unit	Pollock by-product				
	Heads	Liver	Milt	Roe	Viscera
DM, %	97.0	91.6	98.0	99.0	88.7
	%				
OM, %	75.8	99.0	98.5	94.5	95.3
CP, %	69.0	9.1	73.8	80.9	45.7
Crude fat, %	5.9	84.7	25.5	5.4	46.0
GE, kcal/g	4.3	8.4	5.7	5.9	6.9
Amino acid, %					
Essential					
Arg	4.42	0.40	4.61	4.04	2.21
His	1.39	0.10	1.25	1.84	0.64
Ile	2.70	0.29	2.11	4.61	1.38
Leu	4.76	0.54	4.38	7.66	2.47
Lys	5.29	0.27	5.58	6.80	2.32
Met	1.83	0.18	1.26	1.88	0.76
Phe	2.51	0.29	1.93	3.14	1.22
Thr	2.67	0.31	2.70	3.65	1.49
Val	3.19	0.44	3.05	4.99	1.76
Nonessential					
Ala	4.40	0.36	3.48	5.79	1.98
Asp	6.16	0.59	4.06	6.24	2.84
Cys	0.71	0.08	0.65	1.02	0.39
Glu	9.18	0.76	6.55	9.14	4.13
Gly	5.95	0.36	2.97	2.53	2.33
Hydroxylysine	0.20	0.00	0.02	0.00	0.07
Hydroxyproline	0.99	0.03	0.04	0.01	0.23
Lanthionine	0.00	0.00	0.06	0.09	0.02
Orn	0.04	0.01	0.03	0.12	0.03
Pro	3.24	0.26	2.28	4.04	1.52
Ser	2.90	0.27	2.35	4.06	1.44
Tau	0.96	0.08	2.05	0.82	0.87
Trp	0.59	<0.04	0.54	1.00	0.30
Tyr	1.57	0.14	1.88	3.46	1.02
TEAA[1]	28.77	2.83	26.86	38.61	14.23
TNEAA[2]	36.90	2.93	26.96	38.32	17.17
TAA[3]	65.66	5.76	53.82	76.93	31.39
Biogenic amine, μg/g					
Agmatine	0.00	0.00	0.00	0.00	42.60
Tryptamine	100.85	0.00	0.00	0.00	72.44
Phenylethylamine	38.09	0.00	13.73	0.00	0.00
Putrescine	27.56	0.00	152.24	172.86	50.30
Cadaverine	0.00	0.00	0.00	0.00	20.58
Histamine	0.00	0.00	0.00	0.00	Trace
Tyramine	0.00	0.00	0.00	0.00	15.64
Spermidine	0.00	0.00	0.00	Trace	23.79
Spermine	1.29	0.00	0.00	Trace	52.18
Mineral					
Ca, %	5.58	0.00	0.06	0.04	0.20
P, %	3.63	0.17	2.04	1.26	0.60
Mg, %	0.20	0.01	0.10	0.04	0.07
K, %	0.91	0.19	2.09	0.80	0.71
Fe, ppm	62.09	11.36	27.46	26.75	55.89
Zn, ppm	97.99	17.50	66.69	145.63	56.77

[1]TEAA = Total essential AA.
[2]TNEAA = Total nonessential AA.
[3]TAA = Total AA.

The Ca and P concentrations of SMB were the highest (6.76 and 3.93%, respectively) due to the presence of crushed bones. The Fe concentrations ranged from 44.5 to 171.6 ppm. Although K concentrations of ProMega and SMB were similar (0.31%), WFM was higher (0.74%). Zinc concentrations ranged from 75.5 to 239.6 ppm. The presence of bones in SMB and possibly the method of processing the fish meals may explain the difference in mineral profiles noted for these substrates.

Fatty acid concentrations of the 13 fish substrates are reported in Table 6. For the pollock by-products, total fatty acid concentrations ranged from 28 to 443.6

Table 4. Chemical composition of fish protein hydrolysates (DM basis)

Item	Hydrolysate				
	Pollock late	Pink salmon	Red salmon	Salmon protein	Sole
DM, %	95.6	91.5	89.6	96.2	93.4
	%				
OM, %	95.1	94.0	95.0	97.2	91.0
CP, %	80.5	78.2	75.5	92.4	74.7
Crude fat, %	13.8	14.0	18.0	2.2	13.9
GE, kcal/g	5.9	6.0	6.1	4.8	5.7
Amino acid, %					
Essential					
Arg	4.87	4.98	3.96	5.40	4.11
His	1.55	1.46	1.44	1.58	1.56
Ile	3.57	2.96	2.79	2.15	3.25
Leu	6.16	4.95	4.77	4.12	5.42
Lys	6.61	5.14	4.78	5.07	5.71
Met	2.35	1.74	1.73	1.87	2.00
Phe	3.16	2.64	2.58	2.15	2.86
Thr	3.29	2.72	2.64	2.84	3.00
Val	4.09	3.59	3.39	2.71	3.68
Nonessential					
Ala	4.42	3.57	3.47	5.84	3.86
Asp	7.25	5.48	5.34	6.16	6.34
Cys	0.73	0.62	0.66	0.42	0.71
Glu	10.67	7.22	6.96	10.02	8.78
Gly	3.80	3.39	3.43	10.79	3.60
Hydroxylysine	0.00	0.03	0.13	0.42	0.11
Hydroxyproline	0.27	0.22	0.27	2.89	0.33
Lanthionine	0.07	0.14	0.11	0.07	0.41
Orn	0.06	0.08	0.13	0.82	0.07
Pro	2.78	2.46	2.44	5.52	2.52
Ser	3.10	2.42	2.34	3.22	2.76
Tau	0.38	0.37	0.40	1.16	0.41
Trp	0.90	0.80	0.81	0.41	0.85
Tyr	2.74	2.23	2.16	1.42	2.50
TEAA[1]	35.64	30.18	28.09	27.89	31.60
TNEAA[2]	37.15	29.01	28.63	49.17	33.26
TAA[3]	72.79	59.19	56.72	77.06	64.85
Biogenic amine, μg/g					
Agmatine	0.00	0.00	0.00	143.90	0.00
Tryptamine	130.82	117.84	132.19	50.67	0.00
Phenylethylamine	0.00	0.00	0.00	40.19	0.00
Putrescine	102.07	204.91	168.72	177.69	184.65
Cadaverine	135.26	177.78	180.52	785.87	258.88
Histamine	15.86	36.95	23.64	26.67	98.51
Tyramine	87.85	226.27	143.26	411.07	239.22
Spermidine	40.51	145.60	122.12	102.99	0.00
Spermine	21.96	101.82	116.17	27.03	0.00
Mineral					
Ca, %	0.65	0.70	0.53	0.06	1.74
P, %	0.69	1.02	0.78	0.67	0.95
Mg, %	0.10	0.10	0.10	0.07	0.17
K, %	0.50	0.42	0.39	1.86	0.50
Fe, ppm	221.79	688.08	311.67	6.54	368.19
Zn, ppm	107.07	298.29	270.47	56.27	100.93

[1]TEAA = Total essential AA.
[2]TNEAA = Total nonessential AA.
[3]TAA = Total AA.

mg/g (liver). Pollock liver and viscera had the highest concentrations of SFA (160 and 90.5 mg/g, respectively) and MUFA (253 and 124 mg/g, respectively). These 2 by-products are potentially valuable because of their fatty acid content. The omega-3 PUFA are of considerable interest because of their alleged health benefits. These fatty acids are found almost exclusively in aquatic resources (algae, fish, marine mammals) and exist in varying amounts and ratios (Shahidi, 2003). Pollock milt had the highest total PUFA concentration

Table 5. Chemical composition of fish meal substrates (DM basis)

Item	Fish meal		
	White fish meal	Fish protein and marine oil (ProMega)	Salmon meal with crushed bones
DM, %	92.6	91.7	94.6
	%		
OM, %	82.1	96.2	77.0
CP, %	74.6	75.5	57.0
Crude fat, %	9.3	21.8	19.6
GE, kcal/g	4.9	6.3	4.9
Amino acid, %			
Essential			
Arg	4.17	4.28	3.33
His	1.25	1.60	1.30
Ile	2.56	3.22	2.10
Leu	4.43	5.63	3.32
Lys	4.72	6.01	3.37
Met	1.73	2.14	1.52
Phe	2.31	2.93	2.04
Thr	2.47	3.12	2.14
Val	3.02	3.73	2.42
Nonessential			
Ala	4.09	3.86	3.23
Asp	5.62	6.67	4.32
Cys	0.50	0.72	0.44
Glu	8.20	9.24	6.03
Gly	5.72	3.05	5.24
Hydroxylysine	0.19	0.04	0.21
Hydroxyproline	0.94	0.17	1.26
Lanthionine	0.01	0.07	0.07
Orn	0.06	0.06	0.23
Pro	3.13	2.39	3.00
Ser	2.58	2.75	2.13
Tau	0.65	0.08	0.20
Trp	0.63	0.94	0.61
Tyr	1.92	2.59	1.69
TEAA[1]	26.66	32.65	21.54
TNEAA[2]	34.25	32.63	28.65
TAA[3]	60.90	65.28	50.19
Biogenic amine, μg/g			
Agmatine	0.00	0.00	44.61
Tryptamine	159.94	244.31	36.03
Phenylethylamine	20.08	12.58	0.00
Putrescine	64.25	34.71	59.34
Cadaverine	37.21	75.68	221.13
Histamine	14.49	0.00	13.05
Tyramine	37.72	59.11	101.44
Spermidine	37.96	0.00	58.97
Spermine	10.51	10.40	30.40
Mineral			
Ca, %	4.08	0.50	6.76
P, %	2.63	0.53	3.93
Mg, %	0.14	0.10	0.18
K, %	0.74	0.31	0.32
Fe, ppm	125.98	171.55	44.46
Zn, ppm	75.49	101.83	239.62

[1]TEAA = Total essential AA.
[2]TNEAA = Total nonessential AA.
[3]TAA = Total AA.

(44.41 mg/g), and heads the lowest (9.86 mg/g). Liver was next highest, followed by viscera, then roe. Long-chain fatty acids such as eicosapentaenoic acid (**EPA**; 20:5n-3) and docosahexaenoic acid (**DHA**; 22:6n-3) can be produced by chain elongation of linolenic acid (18:3n-3; Reinhart, 1996). The chain elongation of linolenic acid in adult dogs to DHA and EPA is not only slow but nearly nonexistent (Bauer et al., 2004). Therefore, providing the long-chain omega-3 PUFA (EPA and DHA) preformed is more effective than adding linolenic acid to the diet. Pollock milt and roe had the highest concentrations of EPA (19.52 and 10.78 mg/g, respectively) and DHA (17.22 and 9.53 mg/g, respectively). Lower than expected EPA and DHA concentrations were noted in liver and viscera. This may be due to oxidation during storage, even though the samples were kept frozen (–4°C) at all times. Also, liver and viscera samples were collected earlier than others, so were stored for a longer period of time.

Among hydrolysates, red salmon hydrolysate had the highest total fatty acid concentration (126.48) and SPH the lowest (8.33 mg/g). Total SFA concentrations ranged from 2.73 (salmon protein) to 31.76 mg/g (red salmon). Pollock late, pink salmon, and sole hydrolysate had similar total SFA concentrations (average, 23.58 mg/g). Red salmon hydrolysate was highest in total n-3 and n-6 PUFA concentrations (41.24 and 4.74 mg/g, respectively). All other hydrolysates tested had similar n-3 and n-6 PUFA concentrations, except for SPH, which was much below average (2.00 mg/g). Again, all tested hydrolysates had similar EPA (20:5n-3) concentrations (average, 14.18 mg/g), whereas EPA concentration in SPH was much lower (0.40 mg/g). Docosahexaenoic acid (DHA; 22:6n-3) concentration ranged from 0.88 (SPH) to 19.49 mg/g (red salmon hydrolysate). Pink salmon was next highest (16.86), followed by pollock late and sole hydrolysate, which had similar DHA concentrations (average, 11.29 mg/g).

For the fish meal substrates tested, ProMega had the highest total fatty acid concentration (141.67) followed by SMB (129.32), then WFM (66.33 mg/g). Total SFA concentrations ranged from 15.84 (WFM) to 36.05 mg/g (ProMega). Total MUFA concentrations were similar for ProMega and SMB (average, 55.34 mg/g), and lowest for WFM (23.27 mg/g). Salmon meal with crushed bones had the highest PUFA concentration, more specifically the highest arachidonic acid (20:4n-6) concentration (1.99 mg/g). Cats are one of the few species that require a dietary source of arachidonic acid, even when adequate linoleic acid is present in the diet (Case et al., 2000). Cats also need arachidonic acid because of the lack of Δ6 desaturase activity, which is the same enzyme needed to synthesize longer chain omega-3 FA. Fish and fish products are valuable sources of these fatty acids. Eicosapentaenoic acid (20:5n-3) concentrations were consistent among fish meal substrates (average, 12.5 mg/g). Salmon meal with crushed bones had the highest DHA concentration (19.27 mg/g) and WFM the lowest (9.32 mg/g).

Protein quality evaluations of the 13 fish substrates are reported in Table 7. Protein solubility was determined by the potassium hydroxide assay (Araba and

Table 6. Fatty acid concentrations (mg/g) of pollock by-products,[1] fish protein hydrolysates,[2] and fish meal substrates[3]

	Pollock by-product					Hydrolysate					Meal		
Fatty acid	PH	PL	PM	PR	PV	PLH	PSH	RSH	SPH	SH	WFM	ProMega	SMB
DHA[4]	4.05	0.24	17.22	9.53	2.96	11.37	16.86	19.49	0.88	11.22	9.32	14.85	19.27
EPA[5]	3.83	2.91	19.52	10.78	6.81	15.78	11.05	14.02	0.40	15.86	11.88	15.57	10.04
Total SFA[6]	8.48	159.98	24.30	19.14	90.52	24.39	23.36	31.76	2.73	22.98	15.84	36.05	28.68
Total MUFA	9.63	252.59	34.20	17.85	123.95	32.77	35.82	45.09	3.60	34.39	23.27	58.63	52.04
Total n3 PUFA	8.36	5.34	39.17	21.46	11.56	30.71	34.89	41.24	1.54	33.34	23.36	36.77	38.76
Total n6 PUFA	0.5	2.84	2.03	0.97	1.52	1.97	3.91	4.74	0.33	3.99	1.32	5.48	7.27
Total PUFA	9.86	31.06	44.41	23.78	25.69	35.73	41.14	49.63	2.00	40.86	27.22	46.99	48.60
Total fatty acids	27.97	443.64	102.91	60.78	240.17	92.89	100.32	126.48	8.33	98.22	66.33	141.67	129.32

[1]PH = Pollock heads; PL = pollock liver; PM = pollock milt; PR = pollock roe; and PV = pollock viscera.
[2]PLH = Pollock late hydrolysate; PSH = pink salmon hydrolysate; RSH = red salmon hydrolysate; SPH = salmon protein hydrolysate; and SH = sole hydrolysate.
[3]WFM = White fish meal; ProMega = fish protein and marine oil; and SMB = salmon meal with crushed bones.
[4]DHA = Docosahexaenoic acid; 22:6n-3.
[5]EPA = Eicosapentaenoic acid; 20:5n-3.
[6]SFA = Saturated fatty acids.

Dale, 1990), which resulted in a protein solubility index (**PSI**) of the sample and the protein component of the sample. Pollock by-products had variable PSI (sample) values, ranging from 2.5 (liver) to 71.9% (roe). The PSI of the protein component ranged from 27.5 to 88.9%. Roe had the highest protein quality among pollock by-products, and liver the lowest. The PSI of fish protein hydrolysates ranged from 38.8 to 88.7%. The PSI of the protein component ranged from 51.4 to 91.3%. White fish meal, ProMega, and SMB had similar PSI (sample) values (28.1, 20.1, and 29.1%, respectively), and the PSI of the protein component ranged from 26.6 to 51.1%. According to this assay, SPH had the highest protein quality among all fish substrates tested. Experiments with soybean meal have shown that the efficiency ratio for pigs and poultry does not increase with a PSI over 66 to 70% (Araba and Dale, 1990; Parsons et al., 1991). Using this value as a reference, the fish substrates whose protein quality is not compromised are pollock roe and viscera, pollock late hydrolysate, SPH, and sole hydrolysate.

The IDEA as described by Schasteen et al. (2002) is a relatively new in vitro assay that provides Lys digestibility values using an in vitro method that is correlated to in vivo (poultry) Lys digestibility values. The IDEA data are reported in Table 7. The IDEA values for pollock by-products ranged from 0.78 (viscera) to 0.99 (roe) and were used to calculate Lys digestibility. For pollock by-products, as the IDEA value increased, the Lys digestibility values decreased. Pollock viscera

Table 7. Protein quality evaluations of pollock by-products, fish protein hydrolysates, and fish meal substrates

Item	PSI (sample),[1] %	PSI (protein),[2] %	IDEA value[3]	Lysine digestibility (IDEA),[4] %
Pollock heads	27.2	39.4	0.94	72.5
Pollock liver	2.5	27.5	0.93	73.2
Pollock milt	46.4	62.9	0.83	86.3
Pollock roe	71.9	88.9	0.99	62.1
Pollock viscera	31.9	69.8	0.78	89.5
Pollock late hydrolysate	58.8	73.0	0.48	65.3
Pink salmon hydrolysate	43.2	55.2	0.51	71.4
Red salmon hydrolysate	38.8	51.4	0.59	82.9
Salmon protein hydrolysate	88.7	91.3	ND[5]	ND
Sole hydrolysate	56.5	75.6	0.58	81.1
White fish meal	28.1	37.7	0.71	90.4
ProMega	20.1	26.6	0.76	90.2
Salmon meal with crushed bones	29.1	51.1	ND	ND

[1]Protein solubility index of sample based on potassium hydroxide assay.
[2]Protein solubility index of protein in sample based on potassium hydroxide assay.
[3]IDEA = Immobilized digestive enzyme assay.
[4]Lysine digestibility (IDEA) = immobilized digestive enzyme assay value used to calculate lysine digestibility according to the following equation: 194.4 × corrected IDEA value − standard value, where corrected IDEA value = IDEA value multiplied by a standardization factor.
[5]ND = Not determined.

had the highest Lys digestibility (89.5%) and roe the lowest (62.1%). The negative correlation between the IDEA value and Lys digestibility data for pollock by-products may be explained by the use of fish meal as the standard in the calculation of the IDEA value. Also, the protein solubility of pollock by-products varies substantially with the parts of the fish from which they are derived.

Among hydrolysates, IDEA values were positively correlated to Lys digestibility. Red salmon hydrolysate had the highest IDEA value (0.59) and Lys digestibility (82.9%), whereas pollock late hydrolysate had the lowest values (0.48 and 65.3%, respectively). White fish meal and ProMega had similar IDEA and Lys digestibility values (average, 0.74 and 90.3, respectively).

The true AA digestibilities of the 13 fish substrates as determined using cecectomized roosters are summarized in Table 8. For the pollock by-products, mean digestibilities of TEAA, TNEAA, and TAA were not different from each other ($P > 0.05$).

Total AA digestibilities of fish protein hydrolysates were not significantly different ($P > 0.05$) for sole, pink salmon, and red salmon hydrolysates (85.8, 82.7, and 84.2%, respectively), although the digestibilities of the latter 2 hydrolysates were similar to pollock late hydrolysate ($P < 0.05$). Salmon protein hydrolysate had the highest TAA and TEAA digestibilities (average, 94.6%) and pollock late hydrolysate the lowest (average, 79.2%). The TNEAA digestibilities of all hydrolysates were similar, except for SPH, which had a higher TNEAA digestibility (93.3%).

White fish meal, ProMega, and SMB were not different ($P > 0.05$) from each other for TEAA, TNEAA, and TAA digestibilities. Individual AA digestibilities generally followed this same pattern. Notable differences in Asp digestibility were observed among fish meal substrates, with ProMega having the highest digestibility (93.5%) and WFM the lowest (78.4%). The IDEA Lys digestibility data were generated using fish meal as a standard and are highly correlated with in vivo measurements of fish meal substrates such as WFM and ProMega. However, when pollock by-products or hydrolysates were analyzed, IDEA predicted a much lower digestibility than was found in the rooster assay. Pollock roe, heads, and late hydrolysate had greater variation between assays, with Lys digestibility differing by approximately 29, 17, and 9%, respectively. Pollock liver and pink salmon hydrolysate differed by 6.4%. Whereas all of the above fish by-products and hydrolysates displayed noticeable variation between the 2 test methods, pollock milt, pollock viscera, red salmon hydrolysate, and sole hydrolysate had comparable values between assays.

The lower Lys digestibility values predicted in the IDEA assay may be explained by the variable chemical composition of fish by-products and hydrolysates. Also, the protein solubility of fish by-products and hydrolysates tends to vary with the parts of the fish from which they are derived and with the processing technique used to prepare them. The IDEA analysis is being investigated for utility in predicting protein digestibility in fish substrates, and further research is needed. To our knowledge, no previous data have been published using the IDEA assay to determine protein quality of fish substrates.

Pollock by-products have been reported to have CP concentrations ranging from 41.2 to 87.5% (Bechtel, 2003). The CP values for pollock by-products used in the current study, except pollock liver, fell within this range. Bechtel (2003) reported a low fat content of less than 6% for heads, frames, and skin, whereas viscera had a fat content greater than 47%. Fat concentrations in the current study are closest to those reported by Bechtel (2003) except for liver that was much higher (84.7%). Batch variation may be the major reason for the variable fat concentrations.

The PER experiment (Table 9) compared the protein efficiency ratios of SMB and SPH to a whole egg meal control. Data indicated that SMB had high protein quality for chicks (PER= 3.5). High quality proteins have PER values generally greater than 2.0. For example, casein, a standard reference protein, has a PER value of 2.5 (Munro and Allison, 1969). Feeding SPH resulted in a low PER value (1.4). Both feed intake and weight gain of the chicks fed SPH were far below comparable values for the other 2 substrates, resulting in the lower PER value. Salmon protein hydrolysate has an imbalanced AA pattern, limiting the growth potential of the chicks. The ratio of TNEAA:TEAA for SPH was 1.8:1, and SMB had a ratio of 1:1. Also, SPH had an extremely high concentration of Gly (10.79%). The chemical scores for whole egg meal, SMB, and SPH were 86.9, 81.5, and 33.2, respectively, and Trp was determined as the first limiting AA. The chemical score data are in agreement with the PER data, indicating that SPH was not a good quality protein source when fed as the sole source of CP.

The nutritional value of SMB and SPH also was evaluated on the basis of their AA composition using the EAAI. The EAAI values are assigned a maximum of 1.00 and a minimum of 0.01 (Hayashi et al., 1986). Using whole egg meal as the reference protein, SMB and SPH were found to be good protein sources, with EAAI values of 1.0 and 0.9, respectively. Based on the method of Oser (1959), feedstuffs are rated as good quality protein sources when the EAAI is equal or greater than 0.90, useful when around 0.80, and inadequate when below 0.70 (Peñaflorida, 1989). The PER and EAAI assays resulted in different interpretations regarding SPH quality because the EAAI measures the contribution that all essential AA make to the nutritional quality of a protein rather than only those AA in greatest deficit. Comparing SPH and SMB TEAA concentrations, both have similar values (27.89 and 21.54%, respectively), indicating that both are good quality protein sources based on the EAAI.

Palatability results with dogs are presented in Table 10. In Exp. 1, dogs consumed more of the diet containing SPH compared with the control diet ($P < 0.01$), and in

Table 8. True digestibility (%) of AA in pollock by-products, fish protein hydrolysates, and fish meal substrates determined in cecectomized roosters in the precision-fed rooster assay[1]

	Pollock by-product[2]					Hydrolysate[3]					Meal[4]			Pooled
Amino acid	PH	PL	PM	PR	PV	PLH	PSH	RSH	SPH	SH	WFM	ProMega	SMB	SEM
Essential														
Arg	86.1^{de}	98.7^{a}	93.3^{abc}	88.9^{cd}	95.1^{ab}	82.9^{e}	84.1^{de}	88.7^{cd}	96.4^{ab}	89.2^{cd}	89.2^{cd}	88.4^{cde}	92.0^{bc}	0.86
His	82.2^{abcde}	72.4^{fg}	83.8^{abc}	88.4^{ab}	78.3^{cdef}	68.0^{g}	74.0^{efg}	75.2^{defg}	90.4^{a}	78.4^{cdef}	80.3^{bcdef}	83.8^{abc}	82.9^{abcd}	1.20
Ile	94.7^{ab}	91.7^{abcd}	93.6^{abcd}	96.3^{a}	93.8^{abcd}	83.2^{e}	87.7^{de}	87.8^{cde}	95.7^{ab}	89.6^{bcde}	94.2^{abc}	96.1^{a}	92.9^{abcd}	0.80
Leu	94.7^{abc}	99.1^{a}	94.8^{abc}	95.7^{ab}	94.3^{abc}	84.1^{e}	87.6^{de}	88.2^{de}	96.0^{ab}	89.9^{d}	93.6^{bc}	96.3^{ab}	93.2^{bc}	0.76
Lys	89.5^{abc}	79.5^{cde}	87.7^{abcd}	90.9^{ab}	89.6^{abc}	74.2^{e}	77.9^{de}	80.5^{cde}	94.9^{a}	84.1^{bcde}	88.0^{abcd}	85.7^{abcd}	84.6^{bcd}	1.22
Met	94.8^{a}	90.5^{bcd}	94.9^{a}	95.8^{a}	93.9^{ab}	83.9^{e}	87.7^{de}	88.6^{d}	96.5^{a}	89.8^{cd}	93.5^{abc}	96.6^{a}	93.3^{abc}	0.68
Phe	93.8^{ab}	98.2^{a}	94.7^{a}	93.7^{ab}	92.6^{ab}	82.0^{d}	85.1^{cd}	86.1^{cd}	95.6^{a}	88.3^{bc}	92.9^{ab}	95.4^{a}	92.9^{ab}	0.88
Thr	91.3^{ab}	82.8^{cd}	92.8^{a}	92.4^{a}	90.5^{abc}	80.1^{d}	83.5^{bcd}	84.0^{bcd}	94.8^{a}	87.4^{abcd}	90.3^{abc}	94.2^{a}	91.4^{ab}	0.97
Val	91.8^{ab}	85.5^{cd}	93.3^{a}	93.2^{a}	90.0^{abc}	82.0^{d}	85.4^{cd}	85.6^{cd}	94.0^{a}	87.6^{bc}	91.3^{ab}	94.1^{a}	91.4^{ab}	0.72
Nonessential														
Ala	93.2^{ab}	92.4^{abc}	95.0^{a}	95.9^{a}	94.8^{a}	81.7^{d}	86.2^{cd}	86.7^{cd}	96.5^{a}	87.2^{bcd}	92.0^{abc}	95.9^{a}	92.4^{abc}	0.87
Asp	91.1^{ab}	87.1^{ab}	92.7^{a}	93.2^{a}	93.1^{a}	62.4^{e}	72.2^{d}	73.1^{d}	89.1^{ab}	75.7^{cd}	78.4^{cd}	93.5^{a}	83.1^{bc}	1.72
Cys	82.3^{abc}	87.8^{a}	84.1^{ab}	79.6^{abc}	71.9^{bcd}	62.8^{d}	67.6^{cd}	72.9^{abcd}	81.4^{abc}	73.9^{abcd}	77.0^{abcd}	86.8^{ab}	79.8^{abc}	1.70
Glu	93.4^{a}	94.7^{a}	93.3^{a}	94.6^{a}	93.5^{a}	79.5^{d}	83.7^{cd}	85.6^{bc}	95.6^{a}	86.5^{bc}	91.1^{ab}	94.8^{a}	91.0^{ab}	0.92
Pro	89.4^{bc}	99.2^{a}	92.0^{ab}	93.9^{ab}	91.9^{ab}	74.9^{d}	80.5^{d}	83.1^{cd}	95.4^{ab}	83.1^{cd}	89.3^{bc}	93.6^{ab}	92.1^{ab}	1.25
Ser	89.6^{ab}	90.8^{ab}	91.1^{ab}	77.8^{d}	89.3^{ab}	77.3^{d}	80.3^{cd}	81.7^{cd}	94.5^{a}	84.2^{bcd}	87.1^{abc}	94.1^{a}	90.6^{ab}	1.10
Trp	93.6^{a}	58.5^{b}	98.0^{a}	98.7^{a}	99.7^{a}	91.6^{a}	95.7^{a}	95.7^{a}	98.0^{a}	96.1^{a}	96.5^{a}	99.7^{a}	96.4^{a}	2.05
Tyr	93.3^{bcde}	101.5^{a}	95.4^{abc}	95.4^{abcd}	94.6^{abcd}	82.6^{f}	86.3^{ef}	87.6^{def}	95.9^{ab}	88.3^{cdef}	93.2^{bcde}	95.1^{abc}	91.7^{bcde}	0.98
TEAA[5]	91.0^{ab}	88.7^{bcd}	92.1^{ab}	92.8^{ab}	90.9^{ab}	80.0^{e}	83.7^{de}	85.0^{cde}	94.9^{a}	87.2^{bcd}	90.3^{abc}	92.3^{ab}	90.5^{abc}	0.80
TNEAA[5]	90.7^{ab}	89.0^{abc}	92.7^{a}	91.1^{ab}	91.1^{ab}	76.6^{d}	81.6^{cd}	83.3^{bcd}	93.3^{a}	84.4^{bcd}	88.1^{abc}	94.2^{a}	89.6^{abc}	0.98
TAA[5]	90.9^{abc}	88.9^{abcd}	92.4^{ab}	92.0^{ab}	91.0^{abc}	78.4^{e}	82.7^{de}	84.2^{cde}	94.2^{a}	85.8^{bcd}	89.3^{abcd}	93.2^{a}	90.1^{abc}	0.90

[a–f]Means in the same row without common superscript letters differ ($P < 0.05$).
[1]Data are means of 3 roosters.
[2]PH = Pollock heads; PL = Pollock liver; PM = Pollock milt; PR = Pollock roe; and PV = Pollock viscera.
[3]PLH = Pollock late hydrolysate; PSH= pink salmon hydrolysate; RSH = red salmon hydrolysate; SPH = salmon protein hydrolysate; and SH = sole hydrolysate.
[4]WFM = White fish meal; ProMega = fish protein and marine oil; and SMB = salmon meal with crushed bones.
[5]TEAA= Total essential AA; TNEAA = total nonessential AA; and TAA = total AA.

Table 9. Protein efficiency ratio (PER) of chicks fed whole egg meal, salmon meal with crushed bones, and salmon protein hydrolysate, essential AA index (EAAI) using whole egg meal as the reference protein, and chemical score

Treatment[1]	ADG, g·chick^{-1}·d^{-1}	ADFI, g·chick^{-1}·d^{-1}	Protein intake, g·chick^{-1}·d^{-1}	PER	EAAI[2]	Chemical score
WEM	16.8	28.9	2.8	5.8	1.0	86.9
SMB	8.3	23.1	2.3	3.5	1.0	81.5
SPH	1.8	13.0	1.3	1.4	0.9	33.2
SEM[3]	0.5	1.0	0.1	0.1	—	—
LSD	1.5	2.9	0.3	0.4	—	—

[1]WEM = Whole egg meal; SMB = salmon meal with crushed bones; and SPH = salmon protein hydrolysate.

[2]EAAI = $n\sqrt{\prod_{i=1}^{n} \frac{AA_i/TEAA_i}{\text{Egg } AA_i/\text{Egg } TEAA_i}}$, where n = number of essential AA; AA_i = individual essential AA, including Cys and Tyr; and $TEAA_i$ = total essential AA.

[3]Pooled SEM.

Exp. 2, dogs consumed similar amounts of each diet. According to Griffin (2003), an animal's appetite can skew food preference; therefore, consumption alone should not be the deciding factor for food preference. If the dog is hungry, it could eat equal amounts of both diets. In Exp. 1, dogs approached the SPH-containing diet first more often (52.5%) than the control diet. Also, the dogs' first choice for consumption was the SPH diet (70%). First approach and first consumed responses are useful but subjective and, therefore, are not the best indicators of palatability. In addition, first approach and first consumption data often are difficult to measure and the repeatability of these measures is questionable (Griffin, 2003). Intake ratios are the best indicators of overall palatability preference (Trivedi et al., 2000). The IR for the SPH treatment was 0.73. An IR of greater than 0.50 implies a preference for a particular diet. Determination of the corrected IR allowed statistical evaluation of diet preference if different from zero. Corrected IR data are in agreement with IR data, indicating the dogs preferred the SPH-containing diet.

In Exp. 2, dogs approached the diet containing SMB first more often (72.5%) than the control diet. Also, the dogs' first choice for consumption was this diet (62.5%), but the IR for this treatment was 0.52, which indicates that dogs consumed both diets equally. Corrected IR (0.02) data support IR data and indicate no preference in intake between the control diet and the diet containing SMB.

Palatability results can be influenced by several factors, especially flavor, food texture, and size and shape of kibble (Trivedi et al., 2000). The SPH must have provided a taste that the dogs preferred, thus the increase in the IR. Hydrolysates are known to enhance palatability of dog and cat foods (Heinicke, 2003).

Salmon protein hydrolysate was highly palatable and had a high quality protein, even though it had the highest concentration of biogenic amines. Opstvedt et al. (2000) demonstrated that the biogenic amines such as histamine, cadaverine, putrescine, or tyramine did not reduce production performance in salmon. The authors speculated that the reduced growth and feed utilization observed in salmon was associated with the formation of toxic compounds formed during unfavorable storage conditions of the fish prior to processing or during processing fish into fish meal, and was not due to biogenic amines. Also, the biogenic amine content of foodstuffs can be modified during food processing (Kalac and Krausová, 2005). In our experiment, the extrusion pro-

Table 10. Palatability estimates of salmon meal with crushed bones (SMB) and salmon protein hydrolysate (SPH) in dog diets

	Experiment 1[1]			Experiment 2[1]		
Item	Control	SPH	*P*-value	Control	SMB	*P*-value
Amount consumed,[2] g·kg of BW^{-1}·d^{-1}	7.9 ± 2.1	21.2 ± 3.2	<0.01	16.4 ± 2.9	17.7 ± 3.4	0.414
Intake ratio[3]	0.27	0.73	<0.01	0.48	0.52	0.673
Corrected intake ratio[4]	–0.23	0.23	<0.01	–0.02	0.02	0.673

[1]Experimental diets contained 10% of SPH or SMB, and control diets contained 35% of poultry by-product meal.

[2]Values are means of n = 10 dogs/treatment.

[3]Intake ratio = grams SMB or SPH consumed/grams of both diets (SMB or SPH + control) consumed.

[4]Corrected intake ratio = intake ratio – 0.5, to indicate a diet preference, if there was a diet preference, significantly different from zero.

cess may have resulted in some degradation or modification of the biogenic amines in SPH.

As regards the SMB substrate, fish bones are an excellent source of Ca and other minerals, but high dietary ash concentration may compromise diet quality. Indeed, low ash diets are preferred for cats (Aldrich, 2004). A significant effort in diet formulation practices by commercial pet food manufacturers is focused on palatability. If a diet is not well ingested, it does not provide nutrients to the animal. Palatability is influenced by many factors including food texture, composition, ingredients, smell, taste, temperature, past experience of the animal, heat treatment, etc. (Trivedi et al., 2000). The owner's perception of the diet is another important criterion as it strongly determines food repurchase potential. The presence of crushed bones appears to have a negative effect on SMB utilization by dogs.

In the current study, 10% inclusion rates were used for both SMB and SPH. This inclusion rate proved effective while avoiding a fishy smell of the complete diet, a feature discriminated against by dog owners. In cat diets, inclusion rates of SMB and SPH will be most limited by the ash content of the fish substrate.

Of the fish substrates tested, all substrates had a relatively good AA pattern except for pollock liver and viscera. Pollock roe and SPH had the highest protein solubility values, yet SPH had an extremely high Gly concentration, a much higher TNEAA concentration, and a much different ratio of TNEAA:TEAA (1.8:1 vs. 1:1) than the other hydrolysates tested.

From the biogenic amine perspective, concentrations varied markedly among substrates, with SPH having very high values. Yet this substrate was of high nutritional value as determined in a number of the tests and was highly palatable to dogs, dispelling the notion that high biogenic amine concentrations are undesirable. However, it must be noted that the SPH used in the dog palatability experiment had undergone extrusion, which may have decreased or modified biogenic amine concentrations that were only analyzed before extrusion.

From the lipid perspective, the best sources of PUFA were pollock milt, red salmon hydrolysate, and SMB. Pollock liver and viscera had high total fatty acid concentrations and perhaps could serve as effective palatants in pet foods. Salmon protein hydrolysate was not the best fish substrate to provide long chain PUFA as it had the lowest concentrations, along with pollock heads, of all fish substrates tested.

From the mineral perspective, pollock heads, WFM, and SMB had relatively high concentrations of minerals, but their bioavailability is unknown.

In conclusion, chemical composition, protein quality assessments, and palatability tests indicate that fish substrates can differ widely and are affected by the specific part of the fish used to prepare fish protein hydrolysates and meals. Fish substrates have great potential to be used in canned foods and dry extruded kibbles and can provide functions to include provision of essential and nonessential AA and n-3 fatty acids, while providing palatants for complete foods.

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